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Degradation and toxicity of amoxicillin after electron beam irradiation

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1. Introduction

Pharmaceutical and personal care products (PPCPs) have been a major concern in water pollution studies, over the past decade. Among PPCPs, antibiotics are especially important due to their drug resistance building potential. It is estimated that 75% of all consumed antibiotic may be excreted unmetabolized in the domestic sewage wastewater facilities [1]. Amoxicillin is a β-lactam antibiotic, being a modified form of ampicillin, which is used to treat bacterial infections (**Fig. 1**).

Several degradation methodologies for β-lactam antibiotics have been reported, such as sorption, biodegradation, photodegradation, oxidation and irradiation. Electron beam irradiation (EBI) treatment is a green technology, being efficient and safe. The radiolysis of water molecules produces reactive species, as shown in Equation 1, which promote the degradation of organic compounds [2].

The objective of this present paper is to show the possible use of ionizing radiation for the degradation and detoxification of amoxicillin antibiotic, one product frequently released through wastewater.

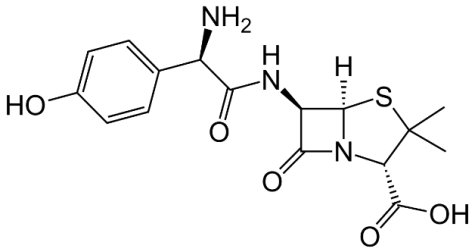

Figure 1 – Molecular structure of amoxicillin

The starting reaction occurring during EB irradiation in liquid wastewater samples is known as water radiolysis and it gives a sequential list of interactions that have been demonstrated by different authors. For instance, when Zhu and coworkers discussed the kinetics, removal mechanisms, degradation products and toxicity during EB irradiation of sulfonamide antibiotics. [5].

$$H_2O \xrightarrow{EB} e^-_{(aq.)} + H^\bullet + HO^\bullet + H_2O_2 + H_2 + H_3O^+ \quad \text{(Eq.1)}$$

Acute toxicity data was determined through the *Vibrio fischeri* reduction of luminescence after being exposed to aqueous solutions of amoxicillin before and after irradiation.

From the literature, we would like to emphasize that for environmental use many advances are in constant development and that EBI and gamma irradiations were applied for the degradation of several pharmaceuticals. Oxacillin results evidenced not only degradation but also reducing antimicrobial activity, altering the biclyclic part among other benefits. Gamma irradiation resulted in > 99% removal

of ciprofloxacin and norfloxacin at 2 kGy (8 kGy.h^{-1} dose rate). [3,4,5,6].

2. Methodology

Irradiation and toxicity of amoxicilin aqueous samples: Standard solution of pure amoxicillin was prepared at 10 ppm. Next step followed the batch scale irradiation that was carried out at an 1.4 MeV electron beam accelerator, through the variation of electric current. *Vibrio fischeri* toxicity assays were applied for measuring acute effects for irradiated and non irradiated samples. In this case the negative effects were related to bacterial luminescence decreasing and associated to the gamma values (relation between lost and remaining light).

Chemical characterization was carried out using Agilent HPLC model 1290 coupled to Sciex QTrap model 3200. Separation conditions were: Restek Ultra Aqueous (150 x 2.1 mm x 3.0 µm) column, mobile phase (A) H_2O + 0.1% formic acid, (B) ACN + 0.1% formic acid, sample injection volume of 5.0 µL. MRM (multiple reaction monitoring) scan type was employed, with Q1 (366.11 Da) and Q3 (208.1 Da). Amoxicillin samples were prepared by diluting standard reagent in ultra-pure water to 10 µg.L^{-1} concentration. Irradiation doses were 0.75, 1.5 and 3.0 kGy.

3. Results and Discussion

Chromatographic results (**Fig. 2**) indicated that peak intensity has dramatically decreased for the irradiated sample (0.75 kGy), from 2,700 to 170 cps. Peak area calculations resulted in a 97.9% degradation rate. The values for samples irradiated at1.5 and 3 kGy are below the detection limit (0.003 µg . mL^{-1})

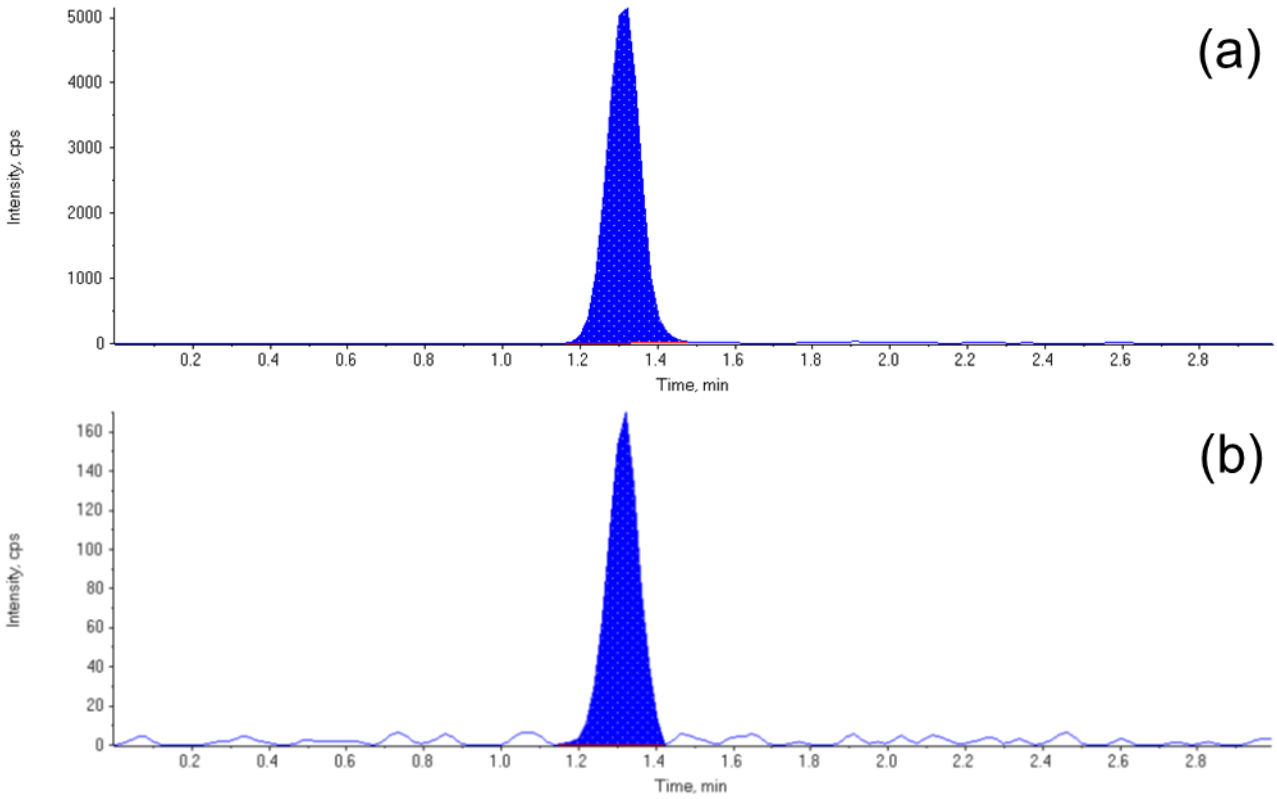

Figure 2 – Comparative chromatogram of non-irradiated (a) and irradiated (b) amoxicillin sample

The same standard solution of amixicillin (10 ppm) were exposed in several dilutions to *V. fischeri* bacteria and the results on toxicity removal were reported at **Table 1**. The average concentration of amoxicillin to

inhibit 50% of luminescence was 10.50, while the 0.75 kGy irradiated solution corresponded to 54.14% of the sample (10ppm). These values indicated 80% of toxicity removal at studied conditions. Note that lower radiation dose (0.75) was more effective than 1.50 and 3.0 kGy for toxicity removal (acute effects). The total organic carbon was slightly reduced at the proposed radiation doses.

Table 1 – Comparative effect of irradiation on mineralization and toxicity assessed by bioluminescent bacteria *V. fischeri* (15 min exposure)

Doses (kGy)	**Total Organic Carbon (TOC) (mg L^{-1})**	**TOC Removal (%)**	**CE50% (15min)**	**Toxicity Removal (%)**
0.00	14.44 ± 0.07	-	10.50 (2.3 – 47.82)	-
0.75	13.85 ± 0.12	4.13 ± 0.80	54.14 (31.36 – 93.46)	80.60
1.50	13.28 ± 0.03	8.03 ± 0.17	33.81 (13.37 – 92.43)	68.94
3.00	13.04 ± 0.05	9.74 ± 0.33	29.39 (12.08 – 71.50)	64.27

The variety of pollutants dissolved into the aquatic environment is increasing for many reasons such as wastewater introduced by WWT facilities, industrial discharges of liquid effluents, solid residues and excessive water usage for diverse applications. Healthcare care products, including pharmaceuticals, have been detected in many parts of globe. Regarding negative effects of antibiotics to aquatic biota, they are associated to many types of pollutants affecting reproduction and behavior of aquatic organisms and also bacterial resistance to antibiotics [3]. Generally the effects of antibiotics on bacteria and micro algae are found 2 to 3 order of magnitude below the toxic values for higher trophic levels. But still if antibiotics exert adverse effects on crustaceans in nature, these effects could be an indirect result of an influence on their food organisms.

4. Conclusions

An important reduction of amoxicillin was achieved at 0.75 kGy, at neutral pH solutions, at the same time that there was an effective reduction on toxicity . Further studies will include the decomposition of antibiotics contained into wastewater samples (spiked).

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